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COMPLETE SPECIFICATION

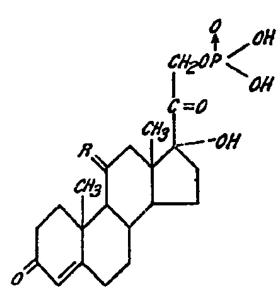
Steroid Compounds

We, MERCK & Co., INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

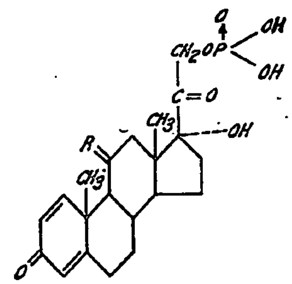
This invention is concerned with water-soluble esters of steroids having cortisone-like anti-inflammatory activity and more particularly with processes for recovering such esters in pure form from reaction mixtures contaminated with inorganic compounds. More specifically, this invention is concerned with purification of the 21-dihydrogen phosphate esters of steroids and their salts, which are water-soluble compounds and which possess cortisone-like anti-inflammatory activity to a marked degree.

The 21-dihydrogen phosphate esters which are amenable to treatment according to the present invention are compounds having one of the following general formulae A and B, in which R is keto or hydrogen plus β-hydroxy, and their therapeutically active nuclearly substituted derivatives as defined in the following paragraph:

Formula A



Formula B



The compounds treated according to this invention may be either unsubstituted compounds conforming to one of the above formulae, or compounds conforming to one of the above formulae except for the presence of one or more substituents selected from 2α-methyl, 6α-methyl, 6β-methyl, 9α-halo (e.g. 9α-fluoro or 9α-chloro), 16α-methyl, and 16α-hdroxy, and possibly of additional olefinic linkages, e.g. in the 6(7) position. Such compounds will in the specification and claims be referred to as compounds "of formula A or B or their derivatives as hereinbefore defined."

Compounds which may be purified according to this invention include the 21-dihydrogen phosphates of cortisone, hydrocortisone, 9α-fluorohydrocortisone, 16α-methylcortisone, prednisone, prednisolone, 9α-fluoroprednisolone, 9α-fluoro-16α-hydroxyprednisolone, 6α-methylprednisone, 6α-methylprednisolone, 16α-methyl-prednisolone, $\Delta^{4,6}$-pregnadiene-11β,17α,21-triol-3,20-dione, and $\Delta^{1,4,6}$-pregnatriene-11β,17α,21-triol-3,20-dione.

The steroid 21-dihydrogen phosphates purified according to this invention, and their alkali metal salts, are outstandingly effective for various purposes because of their water solubility. These compounds are particularly desirable in opththalmic preparations, as the aqueous solutions of these compounds do not cause irritation to the eye due to the presence

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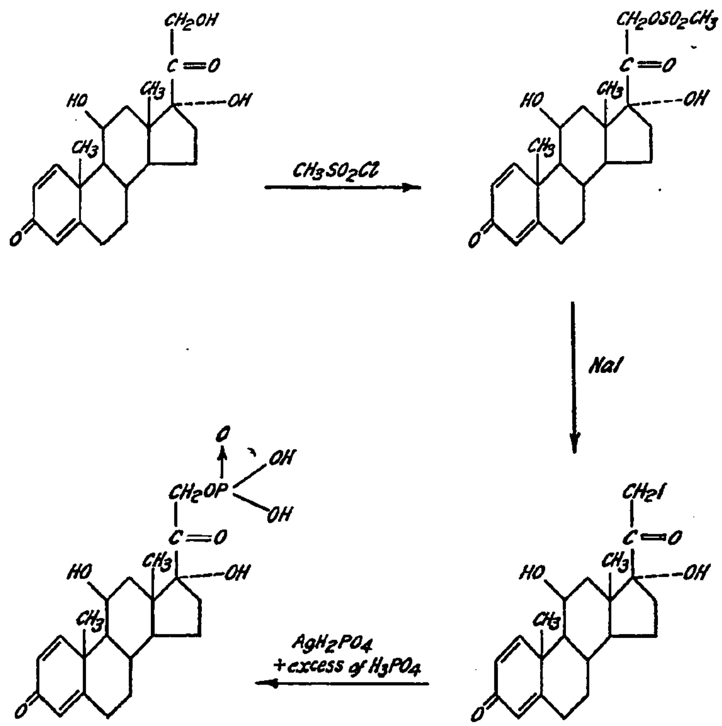
of suspended particles. Aqueous solutions of the steroid 21-dihydrogen phosphates and their alkali metal salts are also highly advantageous for intravenous injection, since adverse reactions to intravenous injection of aqueous solutions are much less common than reactions to organic solvents such as alcohol, which is used as a solvent for water-insoluble steroids injected intravenously.

The 21-dihydrogen phosphate esters may be prepared from the corresponding alcohols, such as cortisone, hydrocortisone, and predniosolone. One method for producing 21-dihydrogen phosphate esters of steroids from the corresponding alcohols consists in reacting the alcohol with methane-sulphonyl chloride to produce the corresponding 21-methanesulphonate, reacting this compound with an alkali metal iodide to produce the corresponding 21-iodo compound and reacting the 21-iodo compound with silver dihydrogen phosphate to produce the 21-dihydrogen phosphate ester. (See, for example, the specification of our copending Application No. 27655/57 (862,383)). This synthesis, using the conversion of prednisolone to 1,4-pregnadiene-11β, 17α-diol-3,20-dione-21-dihydrogen phosphate as an example, may be illustrated by the following equation:



The conversion of the 21-iodo compound, such as 21 - iodo - 1,4 - pregnadiene - 11β, 17α - diol - 3,20 dione in the above equation, to the corresponding 21-dihydrogen phosphate, is carried out in a suitable organic solvent such as acetonitrile. Upon completion of the reaction, water or an organic solvent such as methanol is added to the reaction mixture to dissolve the steroid 21-dihydrogen phosphate which is produced. Water is preferred as it does not dissolve the non-acidic steroids, i.e. unreacted 21-iodo steroid and side products such as 17α,21-dihydroxy steroids and 17α,21-oxido steroids, which may be present in the reaction mixtures. Whether water or an organic solvent is used to dissolve the steroid 21-dihydrogen phosphate, inorganic material, particularly phosphoric acid, is present in the solution. Removal of the inorganic materials is essential in order to obtain the steroid 21-dihydrogen phosphate in pure form acceptable for pharmaceutical use.

The recovery of steroid 21-dihydrogen phosphates from aqueous solutions contaminated with inorganic material has been very difficult prior to this invention. Numerous precipitants, including alkaline-earth metal, polyvalent heavy metal, and amine bases and salts, cannot be used as they coprecipitate steroid phosphate salts and inorganic material. Selective extraction of the steroid phosphate into a suitable organic solvent such as methanol is costly because the water must first be evaporated, generally by freeze-drying.

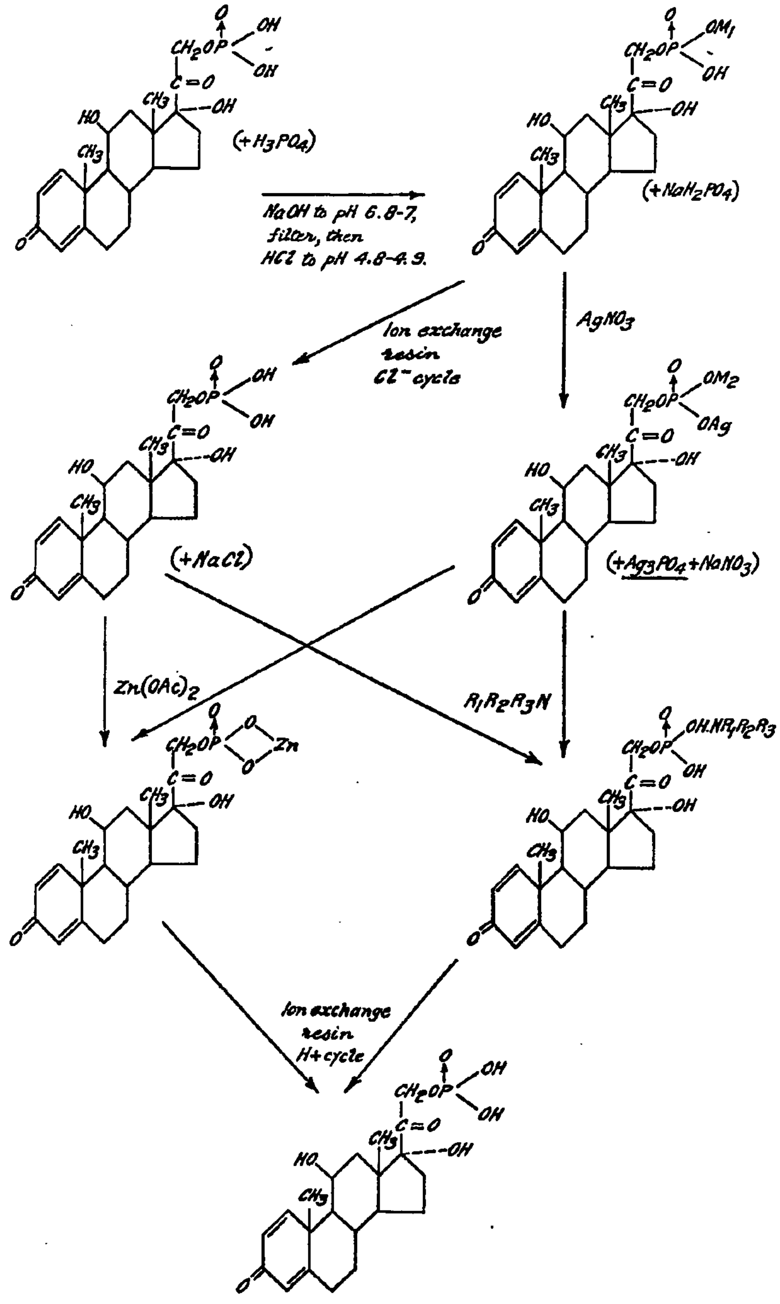
According to the present invention steroid 21-dihydrogen phosphate esters of formula A

or B and their derivatives as hereinbefore defined are recovered in pure form from solutions of them that are contaminated with inorganic phosphate ions by contacting the contaminated solution with an anion-exchange resin or a water-soluble silver salt, both of which form an insoluble substance by reaction selectively with the inorganic phosphate, thereby removing the inorganic phosphate while leaving the steroid 21-dihydrogen phosphate in solution, and separating the resulting solution from the insoluble product.

Some variations in the process are desirable, depending on whether the purification is carried out in aqueous or organic solvent medium. These will be evident in the detailed description which follows.

In a preferred embodiment of this invention, water is added to a reaction mixture containing a steroid 21-dihydrogen phosphate such as prednisolone-21-dihydrogen phosphate, plus inorganic materials such as phosphoric acid, silver iodide and silver phosphate, and non-phosphated steroids such as prednisolone and $17\alpha,21$ - oxido - $\Delta^{1,4}$ - pregnadien - 11β-ol - 3,20 - dione, in an organic reaction medium (preferably acetonitrile), thereby forming an aqueous solution containing the steroid 21-dihydrogen phosphate mixed with phosphoric acid, with the water-insoluble materials, e.g. the steroids, silver iodide and silver phosphate, suspended in it. It is desirable to remove the organic reaction medium by evaporation. The pH is then adjusted approximately to neutrality, and the water-insoluble materials e.g. silver iodide and silver phosphate, are removed by filtration. The pH is then adjusted to about 4.8 to 4.9, which is the optimum pH for removal of inorganic phosphate by reaction with an anion-exchange resin. A common mineral acid such as hydrochloric acid is used, and any precipitate which forms is filtered off. The solution is then contacted with an anion-exchange resin, or alternatively, with a water-soluble silver salt. This removes the inorganic phosphate, i.e., phosphoric acid and sodium dihydrogen phosphate, while leaving the steroid 21-dihydrogen phosphate in solution.

The steroid phosphate solution, after removal of the inorganic phosphate, may then be treated with a zinc salt or suitable amine or amine salt to precipitate the corresponding zinc or amine salt of the steroid phosphate. This separates the steroid from any inorganic material remaining in the solution. The zinc or amine steroid phosphate can then be acidified, preferably with a cation exchange resin, to obtain the pure steroid 21-dihydrogen phosphate. The purification of 21-dihydrogen phosphate esters according to the present invention may be illustrated by the following reaction scheme showing the purification of prednisolone-21-dihydrogen phosphate:



In the above equation M_1 represents a mixture of sodium and hydrogen, the former predominating, and M_2 represents a mixture of silver and hydrogen, the latter predominating, R_1 and R_2 represent hydrogen atoms or organic radicals, and R_3 represents an organic radical.

Weakly basic anion-exchange resins, such as "Amberlite IR—45", "Dowex 3", and "Duolite A—2", are examples of materials suitable for removing inorganic phosphate ions from solution. ("Dowax" and "Amberlite" are registered trade marks). Such resins effect a virtually quantitative removal of inorganic phosphate from solution and do not adsorb the steroid phosphate ester. Strongly basic anion-exchange resins, while usable, are less desirable than the weakly basic anion-exchange resins, as they also adsorb a portion of the steroid phosphate ester while removing inorganic phosphate ions.

The ion-exchange resin may be either on a salt cycle, such as a chloride, acetate, sulphate, or nitrate cycle, or on the hydroxide cycle. The cycle is such that the exchangeable ion of the resin will not cause coprecipitation of non-steroidal material with the steroid phosphate when the latter is precipitated as will hereinafter be described. Chloride and acetate have been found to give the best results.

The pH of the solution treated may be varied widely. Excellent results are obtained when the pH is in the range of about 4 to 8, using an anion exchange resin on a salt cycle such as chloride or acetate. On the other hand, effective separation of steroid phosphate from inorganic phosphate is also obtained by contacting a solution having a low initial pH, for example, about 1 or 2, with an anion-exchange resin in the hydroxide cycle. In a preferred mode of operation, the pH of the solution treated is about 4.8 to 4.9, and the resin is on a salt cycle.

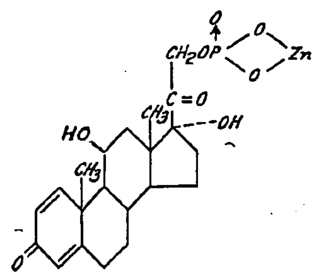
Contact between the solution and the resin may be made in any conventional manner, such as by passing the solution through a bed or column of resin, or slurrying the resin in the solution.

Alternatively, the inorganic phosphate ions may be removed from solution by precipitation. Very few reagents possess the essential requirement of the precipitant, viz. to react selectively with the inorganic phosphate ions and thereby precipitate them while leaving the steroid dihydrogen phosphate ester in solution. The alkaline earth metal salts and the salts of polyvalent heavy metals such as iron, manganese, and zinc, for example, coprecipitate both the steroid dihydrogen phosphates and the inorganic phosphates as the corresponding alkaline- earth metal or heavy metal salts.

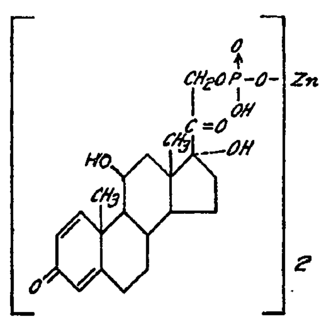
Silver nitrate and other water-soluble silver salts have the surprising property of leaving the steroid 21-dihydrogen phosphate in solution, while precipitating inorganic phosphate ions as trisilver phosphate. Addition of silver nitrate to a solution of a steroid 21-dihydrogen phosphate ester containing inorganic phosphate causes the inorganic phosphate to precipitate as trisilver phosphate, leaving a solution of steroid 21-dihydrogen phosphate containing nitrate ions, from which the steroidal material can be readily separated.

After removal of the inorganic phosphate from solution, the steroid 21-dihydrogen phosphate may be recovered from solution as a water-insoluble salt by adding to the solution a compound that forms an insoluble salt with the steroid 21-dihydrogen phosphate, the compound preferably being a metallic salt. The insoluble salt is preferably precipitated from aqueous or alcoholic solution. An important requisite of the precipitant is that it must precipitate the steroid substantially quantitatively, and it is preferred that the precipitate formed is a salt that is readily converted to the water-soluble steroid 21-dihydrogen phosphate in pure form.

Water-soluble zinc salts such as zinc chloride and zinc acetate have been found to be excellent precipitants. An excess of zinc salt is used to ensure complete precipitation of the steroid. Other zinc salts are also suitable precipitants. The zinc compound reacts with a steroid 21 - dihydrogen phosphate, e.g. prednisone 21-dihydrogen phosphate, hydrocortisone 21-dihydrogen phosphate, or cortisone 21-dihydrogen phosphate, to form the corresponding zinc salt. A typical reaction of this sort is illustrated in the foregoing equation, which shows the conversion of prednisolone 21-dihydrogen phosphate to a corresponding zinc salt. The formula for the zinc prednisolone phosphate, which is indicated



is probably the formula for the precipitate or a major proportion of it. It will be noted that both ionizable hydrogen atoms have been replaced by zinc. However, it is possible that the formula of part or all of the zinc prednisolone is more accurately represented by another formula such as the following formula for an acid salt:



Thus the terms, "zinc prednisolone phosphate" and "a zinc salt of prednisolone 21-dihydrogen phosphate" represent any zinc salt of prednisolone 21-dihydrogen phosphate. The same also holds true for the zinc salts of other steroid 21-dihydrogen phosphates.

The zinc steroid phosphates are readily converted to pure steroid 21-dihydrogen phosphates, as will be hereinafter described. They are also highly effective anti-inflammatory agents which are suitable for incorporation into ointments for topical application. A further use is an anti-inflammatory agents for oral administration.

The water-soluble steroid 21-dihydrogen phosphates may be precipitated as insoluble amine salts rather than as insoluble metal salts, if desired. A typical reaction, the precipitation of prednisolone 21-dihydrogen phosphate, is shown in the equation above. A large number of amines and their acid addition salts are suitable precipitants. When the ultimate product desired is a pure steroid 21-dihydrogen phosphate, the amine chosen is one whose corresponding 21-phosphate is insoluble but readily converted to the pure steroid 21-dihydrogen phosphate. Suitable amines for this purpose include 4,4[1] - diaminodiphenylsulphone, 5 - nitroimidazole, 1 - (3 - aminophenyl) - 3 - methyl - 5 - pyrazolone, 2-amino - 5 - azotoluene, 1 - nitro - 2 - naphthylamine, N - benzyl - β - phenyl - ethylamine, and brucine.

The insoluble amine salts of steroid 21-dihydrogen phosphates are useful as anti-inflammatory agents, particularly in ointments for toptical use. Salts formed by the reaction of a steroid 21-dihydrogen phosphate such as prednisolone 21-dihydrogen phosphate, prednisone 21-dihydrogen phosphate, cortisone 21-dihydrogen phosphate, hydrocortisone 21-dihydrogen phosphate, or 9α-fluoro-hydrocortisone 21-dihydrogen phosphate, with amines such as methylamine, ethylamine, *n*-butylamine, 2-ethylhexylamine, diethylamine, dibutylamine, dimethylamine, triethylamine, hydroxylamine, triethanolamine, *DL*-α-phenylethylamine, aniline, 2,6-lutidine, α-naphthylamine, β - naphthylamine, pyridine, 7 - methylquinoline, acetoguanidine, 2 - amino - 5 - azotoluene, 1 - nitro - 2 - naphthylamine, 2-aminoanthraquinone, 4 - amino - 1 - phenyl-2,3 - dimethylisopyrazolone, 2 - amino - 5-azoanisole, pyrrole, 5 - nitroimidazole, aminoguanidine, 2 - (2 - aminoethylamino) - ethanol, aminoethylethanolamine, 2 - amino - 5 - nitrophenol, 2 - amino - 5 - nitrothiazole, 1 - (3-aminophenyl) 3 - methyl - 5 - pyrazolone, 2 - aminopyrimidine, 4,5 - diaminouracil, 4,4[1]-diaminodiphenylsulphone, betaine, β-alanine, 2 - amino - 3 - carboxylpyridine, phenylhydrazine, 2,4 - dinitrophenylhydrazine, and Girard's "T" reagent, or their acid-addition salts such as the hydrochlorides, possess anti-inflammatory activity. A particularly valuable anti-inflammatory amine salt is the triethylamine salt of prednisolone 21-dihydrogen phosphate.

Pure steroid 21-dihydrogen phosphate esters may be recovered from the insoluble salt by acidification. A preferred means of acidification is the use of a strongly acidic cation-exchange resin in its hydrogen cycle. Various sulphonic-acid resins are among the suitable cation - exchange resins. The steroid 21-phosphate salt in a suitable solvent such as methanol is contacted with the ion-exchange resin. The resulting product is a solution of steroid 21-dihydrogen phosphate. This step is shown in the above equation by way of example as the conversion of prednisolone 21-dihydrogen phosphate zinc salt to prednisolone 21-dihydrogen phosphate.

The steroid dihydrogen phosphate may either be recovered in pure form by conventional means such as evaporation of the solvent or may be converted to an alkali metal salt. Either a monoalkali metal salt, obtained by reacting substantially equimolar amounts of the steroid dihydrogen phosphate and an alkali metal alkoxide, or a dialkali metal salt, may be formed. The conversion to the alkali metal salt is carried out in an anhydrous organic solvent to facilitate the recovery of the pure salt. Suitable solvents include the lower aliphatic alcohols, especially methanol. The alkali metal alkoxides such as sodium methoxide are preferred neutralizing agents, although alkali metal hydroxides may be used. By way of example, monosodium prednisolone 21-hydrogen phosphate is made by neutralizing prednisolone 21-dihydrogen phosphate to a pH of 5.2 to 5.5 with sodium methoxide in methanol solution. The disodium salt is made by neutralizing to a higher pH, about 9.2 to 9.6. Other steroid phosphate alkali metal salts are made in the same manner from the corresponding steroid 21-dihydrogen phosphates. The alkali metal salt my be recovered from the reaction medium by suitable means such as precipitation with ether. An anhydrous organic solvent medium is used instead of an aqueous medium to facilitate recovery of the alkali metal steroid phosphate.

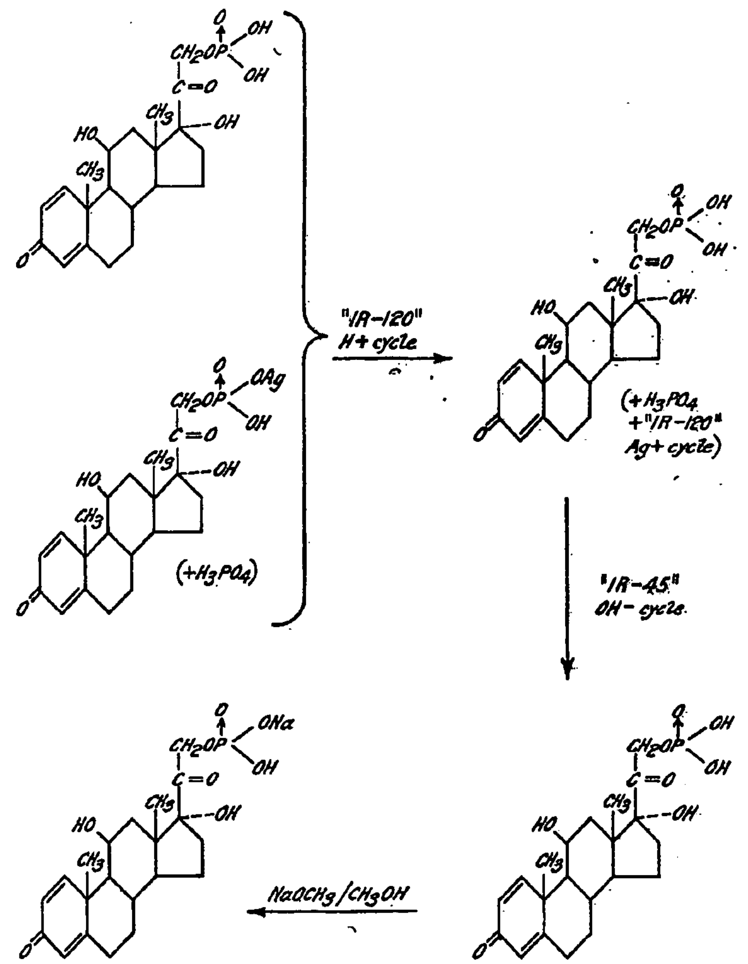
Reaction products may be purified in an organic solvent according to the present invention to obtain pure steroid 21-dihydrogen phosphates. Methanol and the other lower aliphatic alcohols are among the suitable solvents for this purpose. It is possible to use a somewhat simpler purification procedure in an alcoholic solvent than in water. A preferred procedure, described with reference to the purification of prednisolone 21-dihydrogen phosphate but equally applicable to the purification of other steroid 21-dihydrogen phosphates, is as follows:

A reaction product containing prednisolone 21-dihydrogen phosphate and impurities in acetonitrile or other organic reaction medium is diluted with methanol. This dissolves all steroids present, e.g., prednisolone, 21-iodo-$\Delta^{1,4}$ - pregnadiene - 11β,17α - diol - 3,20-dione, and 17α,21 - oxido - $\Delta^{1,4}$ - pregnadien-

11β - ol - 3,20 - dione, as well as prednisolone 21-dihydrogen phosphate, and the silver salt thereof, and phosphoric acid. Silver iodide and silver phosphate are insoluble and may be removed by filtration. The solution is treated with a cation exchange resin, such as "Amberlite IR—120", in its hydrogen form. This removes all silver present, converting the silver salt of prednisolone 21-dihydrogen phosphate to the free acid ester. The effluent is treated with an anion exchange resin, such as "Amberlite IR—45" in its hydroxyl form, to remove inorganic phosphate, which is present chiefly as phosphoric acid. The acetonitrile is then distilled off. This may be done earlier in the process if desired.

Purification in an alcohol is simpler than in water because it is possible to form the sodium salt directly after the inorganic phosphate has been removed, without first forming the zinc or an amine salt. Either the monosodium or disodium salt may be formed by reaction of prednisolone 21-dihydrogen phosphate with sodium methoxide in methanol as previously described.

The equation for the purification of prednisolone 21-dihydrogen phosphate in methanol is as follows:



A typical procedure for preparing steroid 21-dihydrogen phosphate esters from the corresponding alcohols is illustrated in detail below with reference to the conversion of prednisolone to prednisolone 21-dihydrogen phosphate.

PREPARATION OF PREDNISOLONE 21-METHANESULPHONATE

70 l. of dry pyridine and 7.5 Kg. of prednisolone are charged to a 30-gallon jacketed glass-lined still. The mixture is agitated until complete solution is obtained. About 40 l. of pyridine is distilled at high vacuum while maintaining the batch temperature below 40° C. The solution is cooled to 0° C., and 2.2 l. of methanesulphonyl chloride are charged. The batch temperature is maintained between 0° and +3° C. during charging of the methanesulphonyl chloride. An atmosphere of flowing nitrogen is maintained in the still, and

the mixture is agitated during the last stages of the addition. The mixture is then aged for one hour, and 15 gallons of ice water are added cautiously to the still while maintaining the temperature between 0° and 5° C. The still contents are then transferred to a jacketed kettle equipped with an agitator, and 62 kg. of cracked ice in 15 gallons of deionized water are added. The batch is aged one hour and a solution of 2 l. of concentrated (37%) hydrochloric acid in 4 gallons of deionized water is added. The batch is centrifuged and the centrifuge cake washed free of pyridine with deionized water. The centrifuge cake is then vacuum-dried at 50° C. to a moisture content of about 1%, which requires about three days of drying. Yield about 7.77 Kg. (92%).

PREDNISOLONE 21-IODIDE

To a 30-gallon jacketed glass-lined still 64.5 lbs. (31.0 l.) of dimethylformamide are charged by vacuum. The still contents are agitated as 7.74 Kg. of dry (less than 1% moisture) prednisolone 21-methanesulphonate are charged. Then 4.02 Kg. of sodium iodide are charged. The still contents are heated to 57° to 60° C. by means of a steam jacket and held at this temperature for 30 minutes. The batch is cooled to 35° C. and 12 gallons of deionized water are added at the rate of about one gallon per minute. If the solution becomes cloudy, addition of water is interrupted and the mixture agitated for five minutes before resumption of water addition. After all of the water is added, the batch is transferred to a 50-gallon kettle equipped with agitator and an additional 16.7 gallons of dionized water are added. The batch is cooled to 0° to 5° C. and aged for one hour. The batch is filtered and the filter cake washed and vacuum-dried at 30° to 35° C. to a moisture content of less than 1%. Yield about 7.95 Kg. (96%).

PREPARATION OF SILVER DIHYDROGEN PHOSPHATE

To a 100-gallon jacketed glass-lined still, 216 liters of deionized water and 38.6 Kg. of monobasic sodium phosphate are charged. The charge is heated at 60° to 70° C. until complete solution is obtained. A solution of 18.0 Kg. of silver nitrate in 19 liters of water is added. The batch is aged for one hour at 60° to 70° C., cooled to 30° C. and filtered. The filter cake is washed five times with 5 gallons of dionized water, and air-dried on stainless steel trays at 60° C., to a moisture content of 0.5%. The approximate yield of trisilver phosphate is 12.5 Kg. (85%).

To a 5-gallon stainless steel mixing bowl of a Hobart blender ("Hobart" is a registered trade mark), 4.87 l. of 85% phosphoric acid and 11.75 Kg. of trisilver phosphate are added with gentle agitation. The mixing bowl is cooled with cracked ice during the addition. The agitator speed is increased, and the slurry is agitated for 15 minutes. Then 300 ml. of acetonitrile are added and the mixture agitated for five additional minutes. The slurry is then passed twice through a colloid mill and 4.7 l. of acetonitrile are added to the slurry prior to the second pass. This results in finely divided silver dihydrogen phosphate.

PREPARATION OF PREDNISOLONE DIHYDROGEN PHOSPHATE

The slurry of silver dihydrogen phosphate prepared as described above and an additional 64.7 l. of acetonitrile are charged to a 50-gallon, jacketed, stainless steel still equipped with 120-RPM turbine impeller. The charge is agitated and an atmosphere of flowing nitrogen is maintained in the still. Then 7 Kg. of prednisolone 21-iodide and 1.5 Kg. of a diatomaceous earth filter aid are charged to the still, and the contents are heated to gentle reflux (about 84° C.) for 3 hours 15 minutes. The vapor riser of the still is water-cooled during reflux. The batch is cooled to 30° C. and 30 Kg. of cracked ice and 20 l. of water are added. The batch is transferred to a 100-gallon jacketed kettle equipped with an agitator. The still is rinsed with three 5-litre portions of water which are added to the kettle. The pH is adjusted to a value in the range of 6 to 6.5 by the addition of sodium hydroxide solution, while maintaining the batch temperature below 20° C. The batch is then transferred to a 50-gallon stainless steel still and the kettle washed with two 2-litre portions of water which are added to the batch. The acetonitrile is vacuum-evaporated at 30 mm. of mercury and a batch temperature below 30° C. Water at 60° C. is circulated through the jacket of the still to maintain the batch temperature. The end point of the concentration is indicated by a rise in internal temperature, slowing of the distillation rate, and an increase in foaming. When the concentration is completed, any silver compound on the side of the still is scraped down and the pH of the batch adjusted to within the range of 6.8 to 7 by adding about 4 l. of 34% sodium hydroxide solution while the temperature is maintained below 20° C. The batch is stirred for two hours and readjusted to pH 6.8 to 7. The batch is filtered on a 32-inch ceramic pot connected to a vacuum trap. The filter cake, which is essentially silver iodide, is sucked dry, washed with 10 l. of deionized water, transferred to a kettle, and slurried with 30 l. of deionized water. The slurry is refiltered and the filter cake washed with 30 l. of water three additional times, and the filter cake is collected after the last filtration. The filtrates are combined in a 100-gallon glass-lined kettle.

The solution of prednisolone-21-dihydrogen phosphate, prepared as above described, is contaminated with inorganic phosphate. This solution is then treated according to the present invention to recover prednisolone-21-dihydro-

gen phosphate or salt thereof in pure form as illustrated by the following examples:

EXAMPLE 1
Removal of Inorganic Phosphate with Ion-Exchange Resin

To the combined filtrates of prednisolone-21-dihydrogen phosphate containing inorganic phosphate, prepared as described above, enough concentrated hydrochloric acid is added to adjust the pH to 4.8 to 4.9. This requires about 3.5 l. The batch is filtered, and the filter cake washed with two 2-litre portions of water which are added to the filtrate. Half of the filtrate is charged to a column containing 33 gallons of "Amberlite IR—45" ion-exchange resin on the chloride cycle. The discharge rate from the column is adjusted to 3 l. per minute. Approximately the first 20 l. of effluent are discarded. The effluent is then spot-checked with an ultra-violet scanner until a positive ultra-violet test is obtained. At this point the next 30 gallons of effluent is collected in a 150-gallon kettle. Then the next 5 gallons are collected in a carboy and 10 additional gallons in a second carboy. The contents of these two carboys are added to the kettle, provided they contain at least 1 mg/ml of steroid as shown by ultra-violet absorption. The other half of the filtrate is charged to a second column of "IR—45" and collected in the same manner as the first half. The effluent is prednisolone dihydrogen phosphate free of inorganic phosphate ions.

EXAMPLE 2
Preparation of Zinc Prednisolone Phosphate

A sample of the effluent from Example 1 is assayed by ultra-violet to determine steroid content. The effluent is adjusted to pH 5 to 5.2 with about 50 ml. of concentrated hydrochloric acid. A solution of about 3 Kg. of zinc acetate in 8 l. of water is prepared and heated to 70° C. to 75° C. The exact amount of zinc acetate is two equivalents per equivalent of prednisolone dihydrogen phosphate, which corresponds to about equal weights of both materials. The zinc acetate solution is added to the effluent with agitation over a 10-minute period, which results in immediate precipitation of zinc prednisolone phosphate. This addition is carried out in a steam-jacketed vessel. When the addition is complete the vessel is heated through the steam jacket to internal temperature of 60° C. and maintained at this temperature for one hour with the agitator off to increase the particle size of the product. The batch is centrifuged, the mother liquors discarded, and the cake washed until chloride-free. The cake is spun-dry and then dried in a vacuum drier at 60° C. to a moisture content of less than 10%. This requires over three days. Yield about 3.2 Kg. (dry basis).

EXAMPLE 3
Regeneration of Prednisolone Dihydrogen Phosphate

To a 100-gallon still 66.7 l. (116 lbs.) of dry methanol are charged, then 36 l. of ion-exchange resin "Amberlite IR—120" on the hydrogen cycle and 3.2 Kg. of zinc prednisolone phosphate are charged to the still. The batch is agitated for two hours at 20° to 30° C. Then 400 g. of decolorizing charcoal are added to the batch which is stirred for 30 minutes. The batch is filtered and the precipitate washed with methanol until the cake is substantially free of steroid as shown by an ultra-violet scanner. The filtrate is then recharged to the still. The filtrate is prednisolone dihydrogen phosphate in solution.

EXAMPLE 4
Preparation of Monosodium Prednisolone Hydrogen Phosphate

50 l. of methanol are charged to a 20-gallon still. One pound of freshly-opened sodium methoxide is added and the contents agitated under a nitrogen atmosphere until solution is complete. The filtrate from Example 3 is titrated with this sodium methoxide solution to a pH of 5.2 to 5.5, which requires about 30 to 40 l. of sodium methoxide solution. The batch is concentrated to less than 15 liters under vacuum of 29 inches of mercury and batch temperature below 30° C. The concentration is carried out in a 100-gallon still having a jacket surrounding the still pot. Atmospheric steam is circulated through this jacket until the volume of the batch is about 15 gallons; then water at 60° C. is circulated through the jacket. After concentration 360 lbs. of anhydrous ether are added to the batch which is aged for one hour at 15° to 25° C. in a flowing nitrogen atmosphere. The slurry is filtered, and air is excluded during filtration. The still and the filter cake are washed with two 6-liter portions of ether which are added to the filtrate. The filter cake is vacuum dried on stainless steel trays at 25° to 30° C. Approximate yield 2.55 Kg. (about 35% on dry basis).

While the procedure illustrated in Examples 1—4 represents a preferred mode of practising the present invention, other modes are permissible, as is evident from the foregoing specification. Various alternative modes of operation, such as removal of inorganic phosphate with a silver salt instead of an ion-exchange resin, and precipitation of the predniosolone 21-dihydrogen phosphate as an amine salt rather than a zinc salt, will be illustrated in the examples which follow.

EXAMPLE 5
Precipitation of Inorganic Phosphate with Silver Nitrate

To an aqueous solution having a volume of

240 ml. and containing 9.68 mg./ml. of prednisolone 21-dihydrogen phosphate was added to 20.72 g. (10% excess) of silver nitrate dissolved in 20 ml. of water with stirring. A precipitate was formed. The suspension was allowed to stand for one hour, and the precipitate was then filtered and washed. The pH of the filtrate was adjusted to 6.8 with dilute aqueous sodium hydroxide. More silver phosphate precipitated and was filtered off. The filtrate was collected for further treatment to recover pure prednisolone 21-dihydrogen phosphate.

EXAMPLE 6

Preparation of 4,4[1]-Diaminodiphenylsulphone Salt of Prednisolone 21-Dihydrogen Phosphate and its Conversion to Monosodium Salt

A reagent solution of 4,4[1]-diaminodiphenylsulphone hydrochloride was prepared by dissolving 5.00 g. of 4,4[1]-diaminodiphenylsulphone in the minimum quantity of 2.5 *N* hydrochloric acid to effect solution and diluting to 100 ml. with water.

To a solution containing 2.00 g. of partially purified prednisolone-21-dihydrogen phosphate containing 11% by weight of $PO_4\equiv$ and obtained by the process of Example 1 in 100 ml. of water, 5 g. of the diatomaceous earth filter aid sold under the registered trade mark "Supercel" was added. The entire mixture was stirred vigorously with a mechanical stirrer. To this solution 20 ml. of the solution of 4,4[1]-diaminodiphenylsulphone hydrochloride was added drop-wise with stirring. The mixture was stirred vigorously for 30 minutes after the addition of 4,4[1]-diaminodiphenylsulphone was complete. The resulting suspension was filtered and washed thoroughly with water. The filter cake consisted primarily of the filter aid and the 4,4[1]-diaminodiphenylsulphone salt of prednisolone 21-dihydrogen phosphate.

The filter cake was extracted successively with a 50-ml. portion and two 25-ml. portions of methanol. The methanol extracts were combined, stirred for one hour with 5.00 g. of "IR—120" ion-exchange resin on the acid cycle with a mechanical stirrer. The resin was filtered from the solution and washed twice with 10-ml. portions of methanol. These portions were added to the filtrate, which was evaporated to dryness *in vacuo* on a hot water bath. The residue was dissolved in 10 ml. of methanol, and 2.2 ml. of a solution of a *ca.* 5% solution of sodium methoxide in methanol was added to the solution of prednisolone dihydrogen phosphate. The pH was adjusted to 5.7, and 60 ml. of diethyl ether was added to precipitate monosodium prednisolone hydrogen phosphate. The precipitate was filtered and dried overnight in a vacuum desiccator to constant weight. Yield 0.640 g.

EXAMPLE 7

2 ml. of 1% aqueous solution of prednisolone 21-dihydrogen phosphate, obtained by the procedure of Example 1, was introduced into each of 40 test tubes. Each of the 40 amines listed below was then added to a separate test tube. Two drops of each liquid amine and 0.050 g. of each solid amine were added. In the case of the amines introduced as the free amine, two drops of 2.5 *N* hydrochloric acid were added so that the final pH of the solution was about 7. No acid was added where the amine was supplied as an acid addition salt.

The amine reagents were as follows:

1. triethylamine
2. phenylhydrazine
3. triethanolamine titanate
4. 2,6-lutidine
5. pyridine
6. Girard's "T" reagent
7. betaine hydrochloride
8. *n*-butylamine
9. hydroxylamine hydrochloride
10. aniline
11. *dl*-α-phenylethylamine
12. acetoguanamine
13. β-alanine
14. α-naphthylamine
15. 2-ethylhexylamine
16. 2,4-dinitrophenylhydrazine
17. dioctylamine
18. 2-aminoanthraquinone
19. 4-amino-1-phenyl-2,3-dimethylisopyrazolone hydrochloride
20. 2-amino-5-azoanisole
21. 2-amino-5-azotoluene
22. 1-nitro-2-naphthylamine
23. 7-methylquinoline
24. 2-amino-3-carboxylpyridine
25. 2-(2-aminoethylamino)-ethanol
26. aminoethylethanolamine
27. pyrrole
28. aminoguanidine sulphate
29. 5-nitroimidazole
30. diethylaniline
31. 2-amino-5-nitrophenol
32. 2-amino-5-nitrothiazole
33. 1-(*m*-aminophenyl)-3-methyl-5-pyrazole
34. 2-aminopyrimidine
35. β-naphthylamine
36. 4,5-diaminouracil sulphate
37. dibutylamine
38. 4,4[1]-diaminodiphenylsulphone

A precipitate of the amine salt of prednisolone dihydrogen phosphate was formed in each test tube. Only slight amounts of precipitate were formed in the tubes containing 2-amino-pyridine and 3-amino-quinoline.

The purification may be carried out in alcoholic medium as illustrated by the following example:

EXAMPLE 8

Prednisolone 21-dihydrogen phosphate was prepared by combining 8.0 g. of 21 - iodo- $\Delta^{1,4}$ - pregnadiene - $11\beta,17\alpha$ - diol - 3,20-dione with 12.8 g. of trisilver phosphate and 4 ml. of 100% phosphoric acid in 80 ml. of acetonitrile, refluxing for three hours, 10 minutes, and cooling to room temperature. The insoluble material was filtered off and washed with methanol, which was added to the acetonitrile solution. The solution was slurried with 75 ml. of "Amberlite IR—120" resin on the hydrogen cycle, stirred for 90 minutes at room temperature, and the resin removed. The resin was washed with a small volume of methanol. The total volume of solution at this point was 740 ml. Assay of this solution showed 7.5 mg./ml. of inorganic phosphate (as $PO_4\equiv$). Half of this solution was passed over a column of 250 cc. of "Amberlite IR—45" resin on the hydroxyl cycle at a rate equivalent to a contact time of 25 minutes. The column was washed with about 100 ml. of methanol to yield an inorganic phosphate-free solution of prednisolone 21-dihydrogen phosphate.

The effluent was concentrated to 168 ml. To this solution 9.4 ml. of 2% solution of sodium methoxide in methanol was added, raising the pH to 5.2. The solution was concentrated *in vacuo* to a thick slurry, and 280 ml. of anhydrous ether was added. The solution was stirred for one hour at 5° C., filtered, and washed with ether. Yield 2.0 g. (56%) of monosodium salt of prednisolone 21-dihydrogen phosphate; E% (H_2O) 321, → max 2470; E% (MeOH) 314, → max 2425. The product contained 0.5% of inorganic phosphate (as $PO_4\equiv$).

The purification according to the present invention has been described with particular reference to water and methanol as solvents. However, it is understood that the purification may be carried out in other solvents such as ethanol, isopropanol, tetrahydrofuran, acetonitrile, ethyl acetate, and dimethylformamide.

In view of the provisions of Section 9 of the Patents Acts 1949—61, attention is directed to the claims of our prior Patent No. 805,828.

WHAT WE CLAIM IS:—

1. A process for purifying a solution of a steroid 21-dihydrogen phosphate ester of the formula A or B or their derivatives as hereinbefore defined, contaminated with inorganic phosphate ions, which comprises contacting the solution with an anion-exchange resin having an anion exchangeable with the inorganic phosphate ions of the solution, or with a water-soluble silver salt, and separating the steroid 21-dihydrogen phosphate solution, free from inorganic phosphate ions, from the insoluble product.

2. A process as claimed in Claim 1, in which the steroid ester is prednisolone 21-dihydrogen phosphate.

3. A process as claimed in Claim 1 or 2, in which the starting solution of the steroid is an aqueous solution.

4. A process as claimed in Claim 1 or 2 in which the starting solution of steroid is alcoholic.

5. A process as claimed in Claim 4, in which the alcoholic solvent is methanol.

6. A process as claimed in any preceding claim, including the additional step of adding to the solution, after separation of the insoluble product, a compound that forms an insoluble salt with the steroid 21-dihydrogen phosphate, and recovering the steroid 21-phosphate salt thus formed.

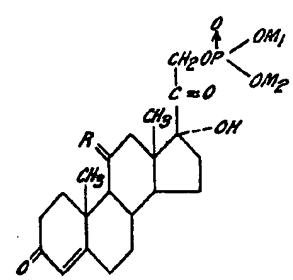
7. A process as claimed in Claim 6, in which the compound that forms an insoluble salt with the steroid 21-dihydrogen phosphate is a metallic salt.

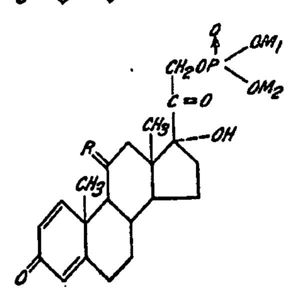
8. A process as claimed in Claim 7, in which the metallic salt is a zinc salt.

9. A process as claimed in Claim 6, in which the compound that forms an insoluble salt with the steroid 21-dihydrogen phosphate is an amine or an amine salt.

10. A process as claimed in any one of Claims 6—9, including the additional step of acidifying the salt and regenerating the steroid 21-dihydrogen phosphate.

11. A process as claimed in any one of Claims 1—5 and 10, including the further step of converting the steroid phosphate ester to an alkali metal salt having one of the general formulae:





and their therapeutically active nuclearly substituted derivatives, in which formulae M_1 is an alkali metal atom, M_2 is a hydrogen atom or an alkali metal atom, and R is $\overset{HO}{\underset{H}{>}}$ or $O=$, by combining the compound having one of the general formulae A and B or its derivative as hereinbefore defined with an alkali metal alkoxide or hydroxide in an anhydrous organic solvent medium, and recovering the steroid-21-dihydrogen phosphate alkali metal salt thus produced.

12. A process as claimed in Claim 11, in which the organic solvent medium is a lower aliphatic alcohol.

13. A process as claimed in Claim 11 or 12, in which the alkali metal alkoxide is a sodium alkoxide.

14. A process as claimed in Claim 13, in which the sodium alkoxide is sodium methoxide.

15. A process as claimed in any one of Claims 11—14, in which the alkali metal alkoxide and steroid 21-hydrogen phosphate are used in approximately equimolar quantities.

16. A process as claimed in any preceding claim for purifying a compound of Formula A or B or its derivative as hereinbefore defined, or a salt of such a compound, substantially as hereinbefore described.

17. A process for purifying a compound of Formula A or B or its derivative as hereinbefore defined, or a salt of such a compound, substantially as hereinbefore described with reference to any one of the foregoing Examples.

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